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A NEW DISINFECTANT TESTING MACHINE.

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In performing laboratory tests to determine the bactericidal power of disinfectants, some of the organisms, living or dead, upon which the disinfecting solutions have been allowed to act, are transferred at stated intervals from the disinfectant solutions to test tubes containing nutrient broth. The bacteria are thus placed in favorable conditions for multiplication, if they have not been killed by the disinfectant solution. As growth will appear in those tubes into which live organisms have been introduced, the results of the tests may be observed and from these observations an opinion formed as to the efficiency of the disinfectant under the conditions of the test. In testing a disinfectant a number of dilutions are used, thus necessitating a number of transfers for each time interval.

It is obvious that in performing these transfers by hand only one transfer can be made at a time; thus the conditions throughout the experiment may constantly be subjected to certain variations in those important factors of time and temperature upon which the uniformity of the tests depends. Moreover, as these transfers must be made in sequence, a single error in technique, disturbing the sequence of manipulation, may ruin the entire experiment. The precise inoculation of a considerable number of tubes at intervals of 15 seconds, as is sometimes required, and in a certain sequence, using different suspensions of bacteria, is a tedious task even if correctly done by an expert in the work. The serial inoculation may well be dispensed with if a method equally satisfactory, as regards the accuracy of the results, can be devised.

In performing disinfectant tests, the use of a machine has a certain obvious advantage over a technique depending wholly upon manual dexterity, in so far as the precision of a mechanical device is substituted for the variations in accuracy of the hand movements of one or more laboratory workers. In addition to this general advantage the machine to be described has certain other definite advantages when contrasted with the hand technique. By the use of the machine, all the transfers due to be made at any particular

time interval are made at the same time and in the same way, thus securing identical conditions for each dilution of the disinfectant or disinfectants used. Furthermore, the machine makes 15 transfers at once, thus doing away with the strain of inoculating a large number of tubes singly in a certain sequence.

It has been recognized that in using a machine greater difficulty as regards the exclusion of air contamination is likely to be encountered; however, this machine has been so designed as to overcome that disadvantage.

Actual comparison of the two methods has shown that the use of the machine greatly simplifies the practice of disinfectant testing.

Construction of the Machine.[1]

A table (T), 31 inches high, 36 inches wide, and 30 inches from front to back, supports the following essential parts of the apparatus: A water bath (B), a large test tube rack (R3), a traveling carriage (Ca), and a flaming device (F.).

The water bath (B) is countersunk, so that half of its depth is below the level of the table top. The external dimensions of the bath are $30\frac{1}{4}$ by 10 by 10 inches; the internal dimensions are $26\frac{1}{2}$ by $5\frac{3}{4}$ by $7\frac{1}{2}$ inches. The space between the outer wooden casing of the bath and its galvanized iron lining (Li), is occupied with sawdust (Sd), for insulation. Two galvanized-iron racks hang in the bath supported at the ends. The first (R1), consists of an angled piece of galvanized iron 26 inches long. To the vertical portion of the rack are riveted 15 brass clips into which fit 15 disinfectant tubes (Td), their bottoms resting on the horizontal portion of the rack. The clips are so arranged that the centers of the tubes are exactly $1\frac{3}{4}$ inches from each other. Clear glass test tubes, 3 inches by $\frac{13}{16}$ inch, are used. They are plugged with cotton and sterilized by dry heat. The second rack consists of three sheets of galvanized iron 26 inches long by $2\frac{1}{2}$ inches wide, the upper two being perforated with a row of 15 holes 1 inch in diameter. Into these fits a row of tubes for the test bacteria (Te), exactly similar to those used in rack No. 1. The tubes in rack No. 2 are so placed that their centers are $1\frac{3}{4}$ inches apart and that they are exactly in line with the corresponding tubes in rack No. 1. The water level (W. L.) in the bath is maintained about an inch below the top of the lining of the bath.

The test tube rack (R3) consists of 3 sheets of galvanized iron, $26\frac{1}{2}$ by $10\frac{1}{2}$ inches, joined together by uprights at the ends. The top of the rack is $3\frac{3}{4}$ inches above the table top and is perforated for test tubes, as is the second sheet, which is secured 3 inches below the top.

[1] The letters refer to similar designations on the illustrations. They are repeated with a complete list of the parts to which they refer at the close of the article.

The bottom sheet rests upon the table top and supports the test tubes. The holes for the test tubes are $\frac{5}{8}$ inch in diameter. They are arranged in six rows of 15 each. In these holes are placed test tubes (B. T.), 6 inches by $\frac{1}{2}$ inch, containing about 7 c.c. of sterile

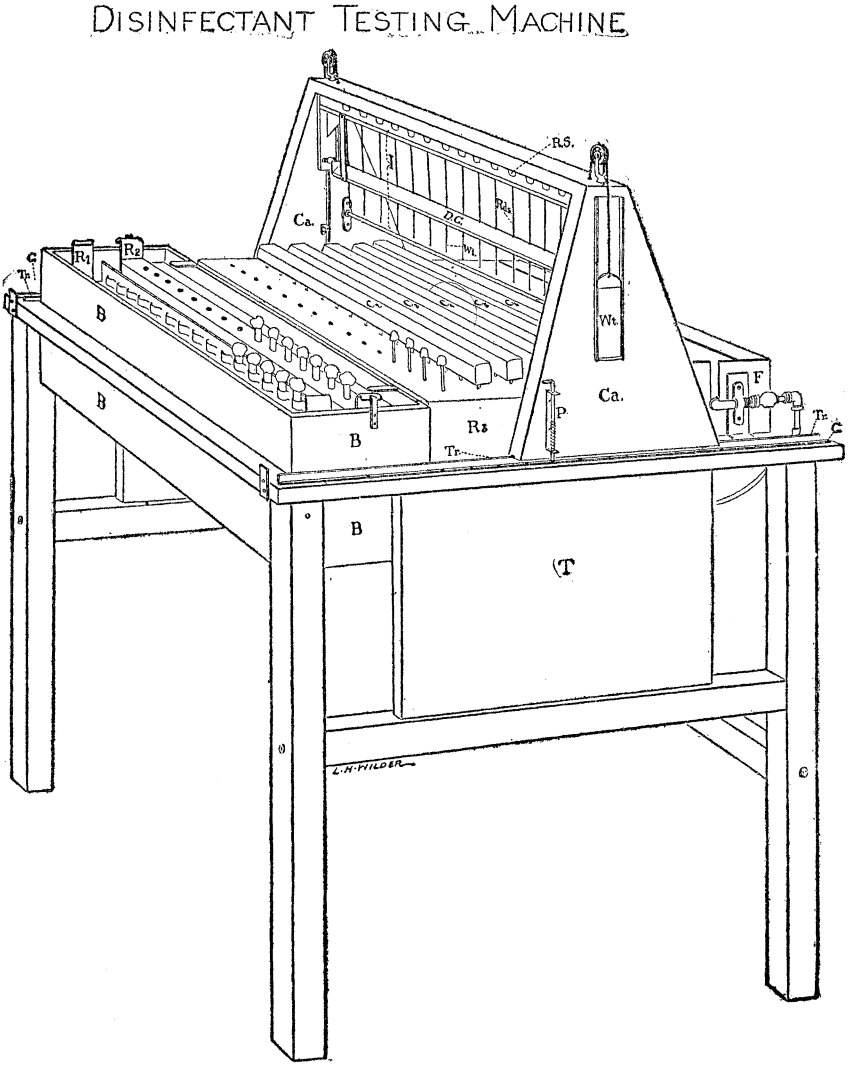

FIG. 1.

broth. The rack is so adjusted on the table by means of guides as to bring the test tubes into alignment with the tubes in rack No. 2. The centers of the tubes in the rows are $1\frac{3}{4}$ inches apart, and the rows are this same distance from each other. When the usual cotton plugs

are removed from the tubes, each row is covered by a galvanized-iron cover (C), previously sterilized by dry heat. The covers are 24 inches long, 1½ inches wide, and 2 inches high, and are open only on

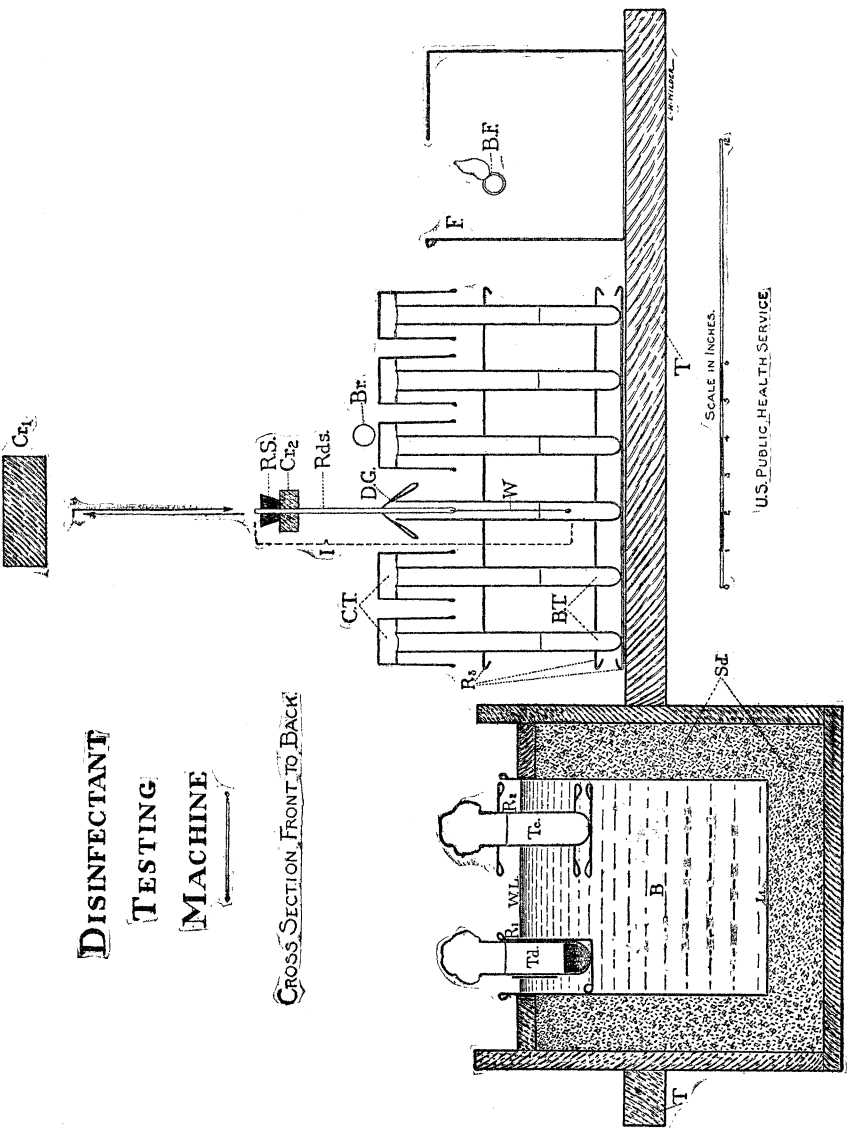

FIG. 2.

their inferior aspect, so as to fit over the rows of tubes. Two small projections serve to secure a layer of cotton (Ct) at the top of the cover inside, thus making them dustproof when applied to the mouths of the test tubes.

The traveling carriage (Ca) consists of two triangular-shaped wooden uprights 16 inches high. These are placed 31 inches apart and are connected with a crosspiece (CR). Small wheels are set into the bottoms of the uprights which rest upon two tracks (Tr), running from the front to the back of the table. Thus the carriage may be moved forward and backward on the table. Pins (P) on the uprights engage in holes in guides (G), running parallel to the tracks and external to them. These holes are so placed that the carriage may be accurately brought to a standstill, so that the center of the crosspiece will be exactly above the various rows of tubes described above. The inoculator (I) is hung from the crosspiece at its ends by pulleys and is so counterbalanced by weights (Wt) that after use the device will always return to its position under the crosspiece (Cr1). The inoculator consists of a crosspiece (Cr2), to the ends of which are attached vertical guides carrying small wheels (2 at each end). The wheels run upon metal tracks on the inner surfaces of the uprights of the carriage, making it possible to raise and lower the inoculator in a plane at right angles with the table top but parallel with the rows of tubes in the racks. This crosspiece is perforated with 15 holes into which fit No. 3 rubber stoppers (RS). The stoppers have one perforation in the center into which No. 8 gauge aluminum rods (Rds) 5½ inches in length are fitted. The lower ends of the rods are flattened and here are attached pieces of No. 23 gauge nichrome wire (Wi). The wires continue downward in line with the rods for 3 inches and terminate in 4 coils, used for transferring the culture after exposure to the disinfectant. They are made of nichrome wire, No. 23 B. & S. gauge.

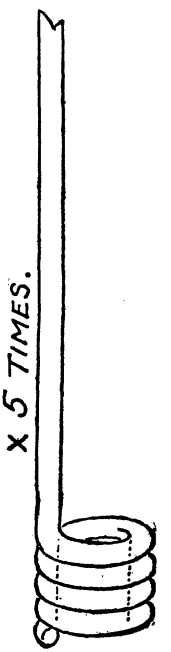

FIG. 3.—Coil for inoculator.

A close cylindrical spiral is made by winding the wire as tightly as possible about a piece of steel or other hard wire having a diameter of 0.072 inch (No. 13 B. & S. gauge). Wind about five full turns, bend the remainder of the wire sharply at a right angle to the wound portion, and parallel to the axis of the cylinder. Remove from the core and cut off the lower end, to leave exactly four complete turns. When completed the successive turns of the spiral must touch one another continuously. The coils are 7¼ inches below the crosspiece of the inoculator.

The centers of the holes in the crosspiece are 1¾ inches apart, and the machine has been so constructed that as the traveling carriage is moved back and forth the centers of the corresponding tubes in the racks will be directly below them. As the wire coils are directly below the centers of the holes in the crosspiece, they are directly

above the centers of the mouths of the test tubes. Two and one-half inches below the crosspiece, and extending entirely across the inoculator, is the dust guard (D. G.). This is attached to the guides of the inoculator and is perforated to allow the aluminum rods to pass through it. It is made of galvanized iron, and in cross section has the shape of an inverted V. An expansion device consisting of a brass cylinder fitting snugly into a metal sleeve serves to connect the dust guard with the guide of the inoculator on the right. It has been so placed as to fit snugly over the mouths of the broth test tubes in rack 3 when the inoculator is lowered. The carriage is strengthened by a brace (Br), which is an iron rod extending across the carriage, and is attached to the side pieces 6½ inches from their bottoms and 2 inches back of the center line. The flaming device (F) consists of a Bunsen burner to which is attached a horizontal brass tube closed at the distal end. The tube is slotted every 1¾ inches, and when the apparatus is lighted the wires of the inoculator can be lowered into the flames (B, F) from these slots and sterilized.

The Performance of Disinfectants—Tests, Using the Machine.

Following is a description of a method for substituting the machine above described for hand manipulations in the testing of disinfectants. It is not our purpose at this time to discuss the technique of disinfectant tests in general, nor to describe them in particular.

In making the tests, the machine should be in a room free from dust and air currents. Before beginning work, the crosspieces of the carriage and inoculator and the internal surfaces of the uprights of the carriage are wiped clean and free from dust. The water bath is filled, and its contents are brought to the desired temperature just before beginning work. The operator now thoroughly flames the interior surface of the dust guard and the rods and wires projecting below. This is conveniently done with an ordinary Bunsen burner attached to a long rubber hose. The flaming device may be conveniently lighted at this time.

The broth tubes are now placed in rack No. 3. Just before putting each tube in its place the stopper is ignited and the mouth of the tube is quickly flamed and the stopper extinguished. When all the broth tubes are in place the stoppers are quickly removed from the row nearest the operator, and this row is covered with its sterile galvanized-iron cover as quickly as possible. The stoppers are removed from the remainder of the tubes and the covers placed on the remaining rows in like manner.

Next, the operator places the tubes containing disinfectant dilutions and the test cultures in racks No. 1 and No. 2, respectively. The cotton stoppers are now removed from the tubes in these racks and the dilutions of disinfectants in the tubes in rack No. 1 are simultaneously poured upon the test cultures in the corresponding

tubes in rack No. 2. This is done by tilting rack No. 1 away from the operator through an arc of 90 degrees.

At the instant of pouring, the time is taken and the experiment begins. The tubes in rack No. 2, now containing various dilutions of disinfectants and organisms, are covered by a semicylindrical galvanized-iron cover, previously flamed, and are gently agitated to mix the contents.

The carriage is now placed in such a position that the coils of the inoculator are over the mouths of the test tubes in rack 2, and after the proper time has elapsed the tubes are uncovered and the inoculator is lowered, immersing the wire coils in the contents of the tubes. The inoculator is raised and the tubes are covered. The operator now removes the cover from the row of broth tubes nearest him and rests it on the crosspiece of the carriage. The carriage is then pushed into such a position that the coils are over this row, the inoculator is lowered, and the coils, bearing organisms from the disinfectant-bacteria suspension, are immersed in the first row of broth tubes. The inoculator is raised, the carriage pushed back to a position over the flaming device, and the cover replaced on the first row of tubes. The inoculator is lowered, bringing the wires and coils into the Bunsen flames, which sterilize them. The operator now raises the inoculator and brings the carriage back to a position over the tubes containing disinfectant and bacteria. When the proper interval has elapsed after the first transfer, the operations described above are repeated, inoculating the 2nd row of broth tubes, and so on until the 3d, 4th, 5th, and 6th rows of tubes have been inoculated, the proper time intervals being carefully observed. The experiment is now completed and rack 3, with tubes and covers in place, is removed from the machine and placed in the incubator.

It has been found advisable to agitate gently the tubes in rack No. 2 just before each transfer is made, and also to immerse and withdraw the wire coils several times from these tubes, repeating this procedure in the broth tubes each time a transfer is made.

Comparison of Machine and Hand Tests.

The following tables illustrate the results of performing disinfectant tests by the two methods, using aliquot parts of dilutions of the same disinfectants. Tests were made within an hour of each other and portions of a single culture were used in each pair of tests.

It will be observed that for the three pairs of tests the results for the 15-minute interval correspond exactly. In the 2½-minute interval there are four pairs of tubes which failed to correspond. In each of these cases the tube planted by machine differs from the one inoculated by hand by but one dilution.

In this comparison the hand test is not taken as a standard of accuracy, as it is quite likely that the machine test represents the more reliable results. It will be noted throughout both tests that the values for the phenol control correspond very closely with the usual values obtained.

The presence or absence of growth in the broth tubes is indicated by plus or minus signs.

TABLE I a.

Disinfectant A.

HYGIENIC LABORATORY PHENOL COEFFICIENT USING MACHINE.

Temperature of experiment, 20° C.
Culture used, *B. typhosus*, 24-hour infusion broth, filtered.
Proportion of culture and disinfectant, 0.1 cc. + 5 cc.

Sample.	Dilution.	Time culture exposed to action of disinfectant for minutes.						Phenol coefficient.
		2½	5	7½	10	12½	15	
Phenol	1-80	−	−	−	−	−	−	
	1-90	−	−	−	−	−	−	
	1-100	+	−	−	−	−	−	
	1-110	+	+	−	−	−	−	
	1-120	+	+	+	+	+	+	$\frac{175}{90}+\frac{350}{110}=$
Disinfectant A	1-150	−	−	−	−	−	−	$\frac{}{2}=$
	1-175	−	−	−	−	−	−	
	1-200	+	−	−	−	−	−	
	1-225	+	−	−	−	−	−	$\frac{1.94+3.18}{2}=$
	1-250	+	−	−	−	−	−	
	1-275	+	+	−	−	−	−	
	1-300	+	+	−	−	−	−	2.56 coefficient.
	1-325	+	+	+	−	−	−	
	1-350	+	+	+	+	−	−	
	1-375	+	+	+	+	+	+	

TABLE I b.

Disinfectant A.

HYGIENIC LABORATORY PHENOL COEFFICIENT USING HAND TECHNIQUE.

Temperature of experiment, 20° C.
Culture used, *B. typhosus*, 24-hour meat infusion broth, filtered.
Proportion of culture and disinfectant, 0.1 cc. + 5 cc.

Sample.	Dilution.	Time culture exposed to action of disinfectant for minutes.						Phenol coefficient.
		2½	5	7½	10	12½	15	
Phenol	1-80	−	−	−	−	−	−	
	1-90	−	−	−	−	−	−	
	1-100	+	+	−	−	−	−	
	1-110	+	+	+	−	−	−	
	1-120	+	+	+	+	+	+	$\frac{200}{90}+\frac{350}{110}=$
Disinfectant A	1-150	−	−	−	−	−	−	$\frac{}{2}=$
	1-175	−	−	−	−	−	−	
	1-200	−	−	−	−	−	−	
	1-225	+	−	−	−	−	−	$\frac{2.22+3.18}{2}=$
	1-250	+	+	−	−	−	−	
	1-275	+	+	+	+	−	−	
	1-300	+	+	+	−	−	−	2.7 coefficient.
	1-325	+	+	+	+	+	−	
	1-350	+	+	+	+	+	−	
	1-375	+	+	+	+	+	+	

TABLE II a.

Disinfectant B.

HYGIENIC LABORATORY PHENOL COEFFICIENT USING MACHINE.

Temperature of experiment, 20° C.
Culture used, *B. typhosus*, 24-hour meat infusion broth.
Proportion of culture and disinfectant, 0.1 cc. + 5 cc.

Sample.	Dilution.	Time culture exposed to action of disinfectant for minutes.						Phenol coefficient.
		2½	5	7½	10	12½	15	
Phenol	1-80	−	−	−	−	−	−	
	1-90	−	−	−	−	−	−	
	1-100	+	+	−	−	−	−	
	1-110	+	+	+	+	−	−	
	1-120	+	+	+	+	−	+	$\frac{\frac{225}{90}+\frac{300}{110}}{2}=$
Disinfectant B	1-150	−	−	−	−	−	−	
	1-175	−	−	−	−	−	−	
	1-200	−	−	−	−	−	−	
	1-225	−	−	−	−	−	−	$\frac{2.5+2.73}{2}=2.61$
	1-250	+	−	−	−	−	−	
	1-275	+	+	−	−	−	−	coefficient.
	1-300	+	+	+	+	−	−	
	1-325	+	+	+	+	+	+	
	1-350	+	+	+	+	+	+	
	1-375	+	+	+	+	+	+	

TABLE II b.

Disinfectant B.

HYGIENIC LABORATORY PHENOL COEFFICIENT USING HAND TECHNIQUE.

Temperature of experiment, 20° C.
Culture used, *B. typhosus* 24-hour meat infusion broth.
Proportion of culture and disinfectant, 0.1 cc. + 5 cc.

Sample.	Dilution.	Time culture exposed to action of disinfectant for minutes.						Phenol coefficient.
		2½	5	7½	10	12½	15	
Phenol	1-80	−	−	−	−	−	−	
	1-90	+	−	−	−	−	−	
	1-100	+	−	+	−	−	−	
	1-110	+	+	+	+	−	−	
	1-120	+	+	+	+	+	+	$\frac{\frac{225}{80}+\frac{300}{110}}{2}=$
Disinfectant B	1-150	−	−	−	−	−	−	
	1-175	−	−	−	−	−	−	
	1-200	−	−	−	−	−	−	
	1-225	−	−	−	−	−	−	
	1-250	+	−	−	−	−	−	$\frac{2.81+2.73}{2}=2.77$
	1-275	+	+	+	+	−	−	
	1-300	+	+	+	+	+	−	coefficient.
	1-325	+	+	+	+	+	+	
	1-350	+	+	+	+	+	+	
	1-375	+	+	+	+	+	+	

TABLE III a.

Disinfectant C.

HYGIENIC LABORATORY PHENOL COEFFICIENT USING MACHINE.

Temperature of experiment, 20° C.
Culture used, B. typhosus, 24-hour meat infusion broth.
Proportion of culture and disinfectant, 0.1 cc.+5 cc.

Samples.	Dilution.	Time culture exposed to action of disinfectant for minutes.						Phenol coefficient.
		2½	5	7½	10	12½	15	
Phenol	1- 80	−	−	−	−	−	−	
	1- 90	−	−	−	−	−	−	
	1-100	+	+	−	−	−	−	
	1-110	+	+	+	+	−	−	$\frac{275+350}{90+110}=\frac{625}{200}=3.12$
	1-120	+	+	+	+	−	+	
Disinfectant C	1-150	−	−	−	−	−	−	
	1-175	−	−	−	−	−	−	
	1-200	−	−	−	−	−	−	
	1-225	−	−	−	−	−	−	$\frac{3.05+3.18}{2}=3.11$
	1-250	−	−	−	−	−	−	
	1-275	−	−	−	−	−	−	
	1-300	+	−	−	−	−	−	3.11 coefficient.
	1-325	+	+	+	−	−	−	
	1-350	+	+	+	+	+	−	
	1-375	+	+	+	+	+	+	

TABLE III b.

Disinfectant C.

HYGIENIC LABORATORY PHENOL COEFFICIENT USING HAND TECHNIQUE.

Temperature of experiment, 20° C.
Culture used B. typhosus, 24-hour meat infusion broth.
Preparation of culture and disinfectant, 0.1 cc.+5 cc.

Samples.	Dilution.	Time culture exposed to action of disinfectant for minutes.						Phenol coefficient.
		2½	5	7½	10	12½	15	
Phenol	1- 80	−	−	−	−	−	−	
	1- 90	+	−	−	−	−	−	
	1-100	+	−	+	−	−	−	
	1-110	+	+	+	+	−	−	$\frac{250+350}{80+110}=\frac{600}{190}=3.15$
	1-120	+	+	+	+	+	+	
Disinfectant C	1-150	−	−	−	−	−	−	
	1-175	−	−	−	−	−	−	
	1-200	−	−	−	−	−	−	
	1-225	−	−	−	−	−	−	$\frac{3.12+3.18}{2}=3.15$
	1-250	−	−	−	−	−	−	
	1-275	+	−	−	−	−	−	
	1-300	+	−	−	−	−	−	3.15 coefficient.
	1-325	+	+	+	+	−	−	
	1-350	+	+	+	+	−	−	
	1-375	+	+	+	+	+	+	

List of Parts of the Machine.

B. =Water bath.
B. F. =Bunsen flame.
Br. =Brace.
B. T. =Broth tubes.
C. =Covers for broth tubes.
Ca. =Carriage.
Cr. 1. =Cross piece of carriage.
Cr. 2. =Cross piece of inoculator.
Ct. =Cotton.
D. G. =Dust guard.
F. =Flaming device.
G. =Guides.
I. =Inoculator.
Li. =Lining of water bath.
P. =Pins.
R. 1. =Rack for disinfectant tubes.
R. 2. =Rack for tubes for test organisms.
R. 3. =Broth tube rack.
R. S. =Rubber stoppers.
Rds. =Rods.
T. =Table.
Td. =Disinfectant tubes.
Te. =Tubes for test organisms.
Tr. =Tracks.
Sd. =Sawdust.
Wi. =Wires.
W. L. =Water level.
Wt. =Weight.